RESEARCH ARTICLE

Development of Twin Model for the Bioremediation of Crude Oil Sludge Contaminated Soil

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ABSTRACT: Twin mathematical models were developed for substrate reduction and biomass increase respectively. The analytical solution of the twin models gave a sigmoidal substrate decline curve and a sigmoidal biomass increase curve respectively. The developed models aided the monitoring and prediction of crude oil sludge degradation in soil by the activities of stimulated biomass. The solution of the twin models when subjected to turning points was useful in determining the biomass daily rate and the biomass peak time.

KEYWORDS: Bioremediation, Crude Oil Sludge, Substrate, Biomass, Modeling, Curve fitting

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INTRODUCTION

Hydrocarbon exploration activities orchestrated by rapid industrialization of crude oil producing countries like Nigeria has resulted in the grandiose pollution of the environment with diverse consequences on human health and the ecosystem. Pollution by crude oil and its components have become a problem of enormous magnitude worldwide [1].

Enormous quantities of crude oil sludge are being generated during crude oil production from oil wells, cleaning of crude oil storage tanks, processes of crude oil refining and separation processes at crude oil ocean terminals before export [2-4]. Several components of crude oil sludge are toxic, mutagenic and carcinogenic [5-7].

According to USEPA (1986), crude oil sludge has been classified as a dangerous environmental pollutant by the United States Environmental Protection Agency [8]. In Nigeria, crude oil sludge has been designated as hazardous pollutant by the Department of Petroleum Recourses DPR [9].Crude oil sludge therefore poses a big threat to the environment as well as to mankind [10]. Crude oil sludge when inadequately disposed to the environment, contaminates the soil, causing loss of the soil fertility nutrients which leads to stunted growth of plants and over a passage of time pollutes the underlying groundwater environment [11]. The environmental impact of crude oil sludge is a major problem facing the people of the Niger Delta region who are predominantly farmers and fishers [12].

It is therefore absolutely imperative that crude oil sludge be treated to avert its environmental hazards. Several physical and chemical mitigation methods have been documented for the cleanup of crude oil sludge contaminated soils; such as incineration, solvent extraction, chemical oxidation, soil washing, scooping and thermal desorption [13-15]. It is important to note that these physicochemical methods of crude oil sludge impacted soil mitigation are very expensive and not environmentally friendly, due to hazards associated with chemical additives and they do not completely remove the pollutants [16]. It is therefore against this backdrop that the need for a cheap, economic, and environmentally friendly mitigation approach to decontaminate crude oil sludge polluted soil has become absolutely inevitable. Environmentally friendly mitigation approach based on biological treatment of petroleum hydrocarbon wastes which gives a reliable, simple and cheap technology is preferred over the conventional physicochemical methods [17].

Bioremediation is a prominent method of cleaning hydrocarbon impacted soils by employing the degradative capabilities of microorganisms which accelerates the rate of

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the substrate degradation by subduing those factors that retard hydrocarbon degradation activities [18]. Heterotrophic bacteria and fungi have demonstrated high degradative capabilities to metabolize hydrocarbon pollutants in soil [19].

Bioremediation of a petroleum hydrocarbon polluted soil is the use of microorganisms such as bacteria, fungi, algae and green plants to decontaminate, remove, degrade, mineralize, transform and detoxify the environmental pollutants into a less toxic forms that is harmless to the environment [20-21].The main aim of the research is to study the impact of crude oil sludge and its bioremediation mitigation in a Niger Delta environment. The study will develop a twin model for substrate reduction and biomass increase that can monitor and predict the extent of soil bioremediation at any time.

MATERIALS AND METHODS

The major materials are soil, crude oil sludge, NPK fertilizer, COW DUNG and moringa leaf powder. The soil was collected from a hydrocarbon unimpacted area of Bodo Community in Gokana Local Government Area of Rivers State, Nigeria. The soil was collected at a depth of 20cm at different areas of the sampling site, as the highest concentration of organic matter and microorganisms are found in this layer [22]. The collected oil sample in a 50kg raffia grade bag was then transported to the experimental set-up site. Crude oil sludge sample was collected from the Nigerian Agip oil company flow station at Oshie town, Ahoada West Local Government Area of Rivers State, Nigeria. The sludge was gotten from the process of crude oil storage tank periodic cleaning. The collected crude oil sludge sample in three twenty-liter containers were then transported to the experimental set-up site. NPK (15:15:15) fertilizer sample, sealed in a 50kg raffia bag was purchased from the Agricultural Development Program (ADP) office in Rumuodamaya, Obio/Akpor Local Government Area of Rivers State, Nigeria. The NPK sample was then transported to the experimental set-up site. COW DUNG sample was collected from the former Port Harcourt Central Abattoir, Oginiba, Trans-Amadi industrial layout, Port Harcourt, Rivers State, Nigeria. The collected Cowdung sample in a 25kg raffia bag was transported to the experimental set-up site. Moringa leaf powder sample was purchased from the Rivers State University farm, Nkpolu-Oroworukwo, Port Harcourt. The Moringa Leaf Powder sample sealed in a 25kg biaxial oriented polypropylene (BOPP) bag was transported to the experimental set-up site. At the experimental site, 200g of the soil sample, 200ml of the crude oil sludge sample, 200g of COW DUNG sample and 200g of the Moringa Leaf Powder sample were collected in clean polyethylene terephthalate (PET) bottles and taken to AUSTINO Research and analysis Laboratory, 2 UPTH Road, off East West Road, Port Harcourt, for initial physicochemical parameters characterization before the experiment.

EXPERIMENTAL PROCEDURE

The experiment studied the bioremediation of 1kg of crude oil sludge mixed in 2kg soil for a period of 92days using NPK fertilizer, COW DUNG (CD) and Moringa Leaf Powder (MLP) as biostimulants. The experiment was carried out in eleven (7litres capacity) buckets labeled, $E_1, E_2, E_3, E_4, E_5, E_6, E_7, E_8, E_9, E_{10}, E_{31}$.

The biostimulants used were combined in varying proportions as described below:

$E_1 = PS + 0.25kgNPK + 0.25kgCD + 0.25kgMLP$

$E_2 = PS + 0.50kgNPK + 0.25kgCD + 0.0kgMLP$

$E_3 = PS + 0.50kgNPK + 0.0kgCD + 0.25kgMLP$

$E_4 = PS + 0.75kgNPK + 0.0kgCD + 0.0kgMLP$

$E_5 = PS + 0.25kgNPK + 0.50kgCD + 0.0kgMLP$

$E_6 = PS + 0.0kgNPK + 0.50kgCD + 0.25kgMLP$

$E_7 = PS + 0.0kgNPK + 0.75kgCD + 0.0kgMLP$

$E_8 = PS + 0.25kgNPK + 0.0kgCD + 0.50kgMLP$

$E_9 = PS + 0.0kgNPK + 0.25kgCD + 0.50kgMLP$

$E_{10} = PS + 0.0kgNPK + 0.0kgCD + 0.75kgMLP$

$E_{31} = PS + 0.0kgNPK + 0.0kgCD + 0.0kgMLP$

The polluted soils (PS) being studied were combined with the biostimulant(s) to stimulate degradation of the crude oil sludge. In experiment E_1, all the biositmulants were combined in equal proportion of 0.25kg each. In experiment E_2, NPK was doubled, cowdung remained 0.25kg and moringa leaf powder was not added. In experiment E_3, NPK remained 0.50kg, cowdung was not added and moringa leaf powder was 0.25kg. In experiment E_4, NPK alone was applied while Cowdung and moringa leaf powder were left out. Experiment $E_5$to E_{10} followed same sequence of combination as E_1 to E_4. In experiment E_{31}, No biostimulants was added to the polluted soil which proceeded on natural attenuation and served as the control experiment.

The soil in the buckets, mixed with 1kg of crude oil sludge, were properly tilled and homogenized, after which samples were drawn at intervals (days 4, 8, 22, 36, 50, 64, 78, 92) for analysis to estimate the extent of degradation. The polluted soil samples prior to sample collections are tilled and homogenized.

Twin Model Development

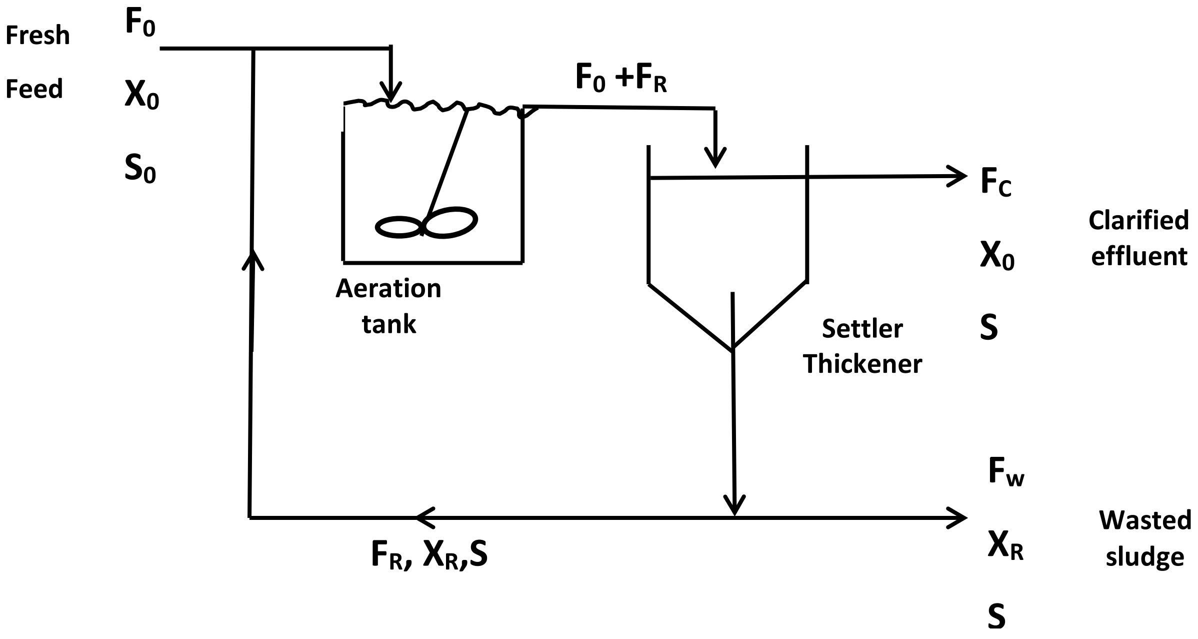

Figure1: CSTR with settler – thickener and recycle of biomass.

The material balance for this process is:

$$\begin{bmatrix}\textit{material in}\\ \textit{with freshfeeds}\end{bmatrix}+\begin{bmatrix}\textit{material in}\\ \textit{with recycle stream}\end{bmatrix}+\begin{bmatrix}\textit{formation by}\\ \textit{biochemical reaction}\end{bmatrix}-\begin{bmatrix}\textit{material output}\\ \textit{to separator}\end{bmatrix} = \text{Accumulation} \qquad (1)$$

So that for the substrate:

$$F_0S_0 + F_R\text{S} + R_SV - - (F_0 + F_R)\text{S} = V\frac{dS}{dt} \qquad (2)$$

And for the biomass:

$$F_0X_0 + F_RX_R + R_x\text{V} - (F_0 + F_R)\text{X} = V\frac{dx}{dt} \qquad (3)$$

Where:

F_0, F_R + is the volumetric flowrate of feed and volumetric flow rate of recycle stream, respectively.
R_S, R_X is the rate of substrate and biomass reaction respectively.
S_0, S is the initial substrate and final substrate concentrations respectively.
X_0, X is the initial biomas and final biomas concentrations respectively. V is the volume of the tank.

When we realize that there is no flow in and out of this work, the first, second and fourth terms of equations (2) and (3) cancel out.

$$R_SV \;=\; V\frac{ds}{dt} \qquad (4)$$

$$R_X V = V\frac{dx}{dt} \quad (5)$$

Again when realize that this work was done on constant volume, equations (4) and (5) reduce as follows:

$$\frac{ds}{dt} = R_S \quad (6)$$

$$\frac{dx}{dt} = R_x \quad (7)$$

Note that, the rate of change of substrate is directly proportional to the decrease of the substrate, while the rate of change of biomass is directly proportional to the biomass.

That is

$$R_S = -bS \quad (8)$$

$$\text{and} \quad R_X = ax \quad (9)$$

Substituting back into equations (6) and (7) gives:

$$\frac{ds}{dt} = -bS \quad (10)$$

$$\frac{ds}{dt} = ax \quad (11)$$

The rate of any inhibitor inhibiting the reaction is directly proportional to the square of material concentration and acts in opposition to the reaction direction. That is:

$$R_{IS} = CS^2 = bkS^2 \quad (12)$$

$$\text{and} \quad R_{IX} = dx^2 = -afx^2 \quad (13)$$

Note that the product of constants is a constant.

Substituting equation (12) and (13) into equations (10) and (11) gives

$$\frac{ds}{dt} = -bS + bkS^2 \quad (14)$$

$$\frac{dX}{dt} = \alpha x - afx^2 \quad (15)$$

If b = $\propto$, k = β, ɑ = ɑ, and f = b. We can therefore rewrite equations (14) and (15):

$$\frac{ds}{dt} = -\propto S + \propto \beta S^2 \quad (16)$$

$$\frac{ds}{dt} = ax - abx^2 \quad (17)$$

Equations (16) and (17) are the required twin model equations for substrate reduction and biomass increase. The analytical solution of equations (16) and (17) give a sigmoidal substrate decline curve and a sigmoidal biomass increase curve respectively, as :

$$S_t = \frac{S_0 e^{-\propto t}}{1-\beta S_0(1-e^{\propto t})} \quad (18)$$

and

$$x_t = \frac{X_0 e^{\propto t}}{1-bX_0(1-e^{\propto t})} \quad (19)$$

With trough and peak equations as

$$DS = \frac{ds}{dt} = \frac{\propto S_0(\beta S_0 - 1)e^{-\propto t}}{[1-\beta S_0(1-e^{-\propto t})]^2} \quad (20)$$

$$Dx = \frac{dx}{dt} = \frac{ax_0(1-bx_0 -)e^{at}}{[1-bx_0(1-e^{at})]^2} \quad (21)$$

Also, trough and peak time at:

$$\frac{d^2s}{dt^2} = 0 \text{ as } t_{\min} = \frac{1}{\propto}\,\text{In}\,\frac{\beta S_0}{(1-\beta S_0)} \quad (22)$$

$$\frac{d^2x}{dt^2} = 0 \text{ as } t_{pk} = \frac{1}{a}\,\text{In}\,\frac{(1-bx_0)}{bx_0} \quad (23)$$

In this work, twin models of substrate reduction and biomass increase have been established for the first time. However, equation (20) and (22) are mathematically correct expressions but sensibly wrong, as there can never be substrate reduction trough and minimum trough time. That is, there will never be a time when substrate reduction curve will change and become substrate increase curve, or, put it the other way, a substrate that is continuously being reduced by the attack of microbes cannot, all of a sudden, begin to increase. The phenomenon is sensibly wrong! So, equations (20) and (22) are mathematical niceties or fineries for this work.

RESULTS AND DISCUSSION

Figure 2 and 3 shows the twin model (substrate reduction) curve fitting profile on the scatter diagram of TPHE1versus time. Figure 2 shows how the model fits the scatter diagram within the experimental data and gives an R^2 of 0.9794. While Figure 3 is a predictive model with the same R^2 but shoots into the future to show the scenario far outside the experimental results and predicts 1243 days (3.4years) for the remediation to reach DPR recommended value of 1000 mg/kg limit of TPH in soil. The coefficients and statistical goodness of fit are as shown in Table 1 below.

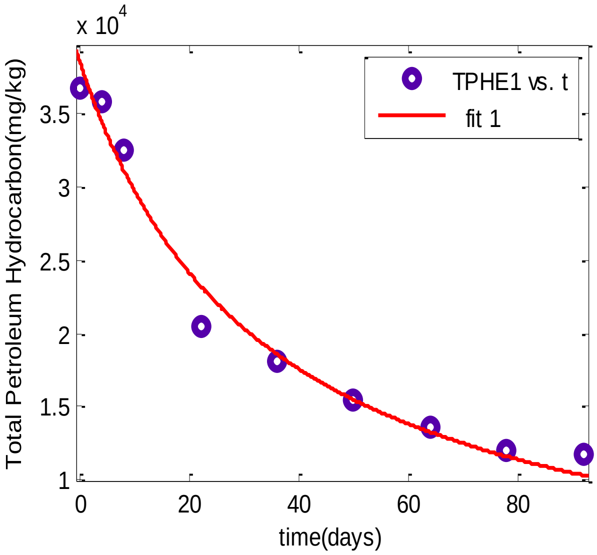

Fig 2 Total Petroleum Hydrocarbon (E1) vs time

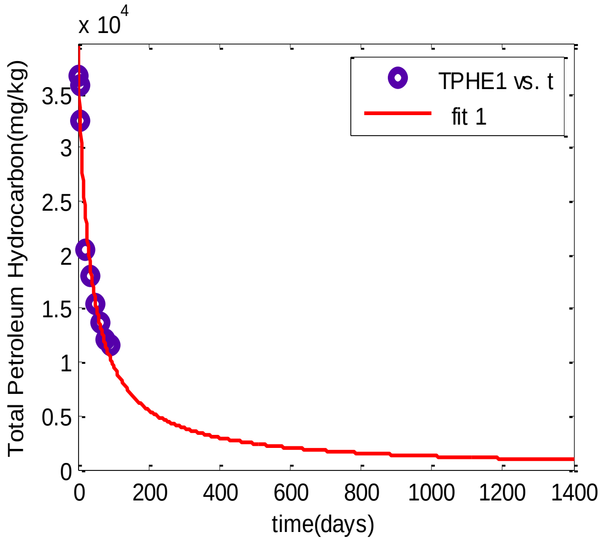

Fig 3: Predictive total petroleum hydrocarbon (E1) vs time

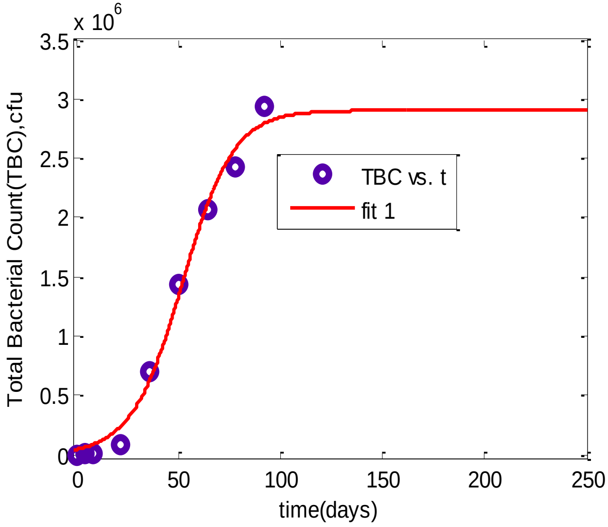

Fig 4: Total Bacterial Count versus time (E1)

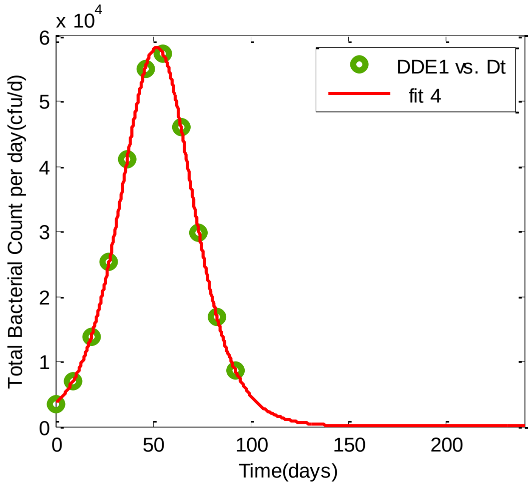

Fig 5: Total Bacterial Count versus time (E1)

Table 1: Coefficients and Statistical Goodness of Fit for TPHE1 to TPHE10

Experiment	**R^2**	**R^2Adj**	**Time at 1000mg/kg**
TPHE1	0.9794	0.9670	1243days = 3.4 years
TPHE2	0.9841	0.9746	1361days = 3.73year
TPHE3	0.9772	0.9635	1513days = 4.15years
TPHE4	0.9809	0.9694	2350days = 6.44years
TPHE5	0.9907	0.9851	1760days = 4.82years
TPHE6	0.9783	0.9653	1960days = 5.37years
TPHE7	0.9753	0.9604	2710days = 7.43years
TPHE8	0.9872	0.9795	1840days = 5.04years
TPHE9	0.9909	0.9854	2400days = 6.58years
TPHE10	0.9707	0.9531	2770days = 7.60years
TPHE31	0.9764	0.9622	Predict

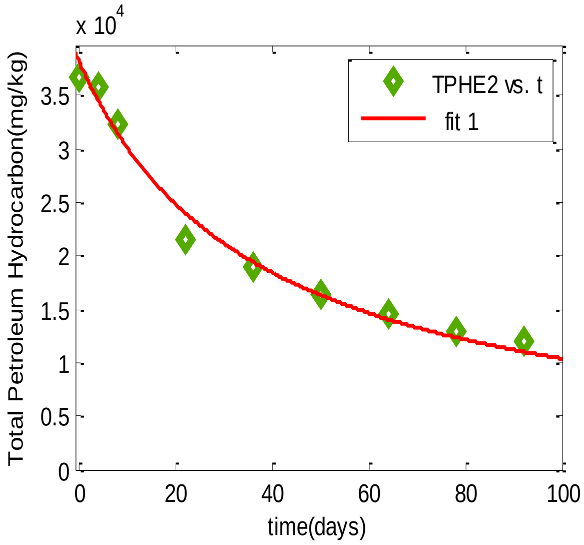

Fig 6: Total Petroleum Hydrocarbon (E2) vs time

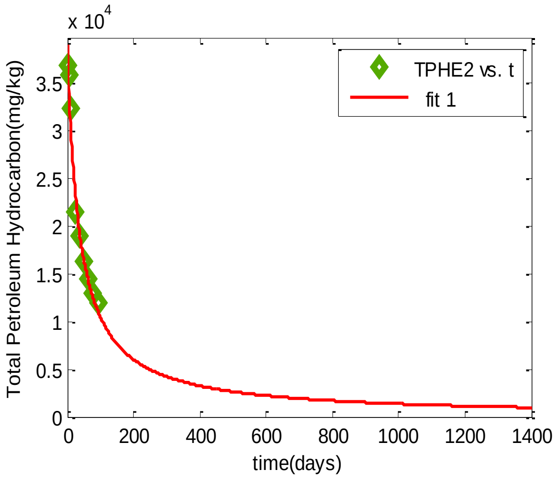

Fig 7: Predictive Total Petroleum Hydrocarbon (E2) vs time

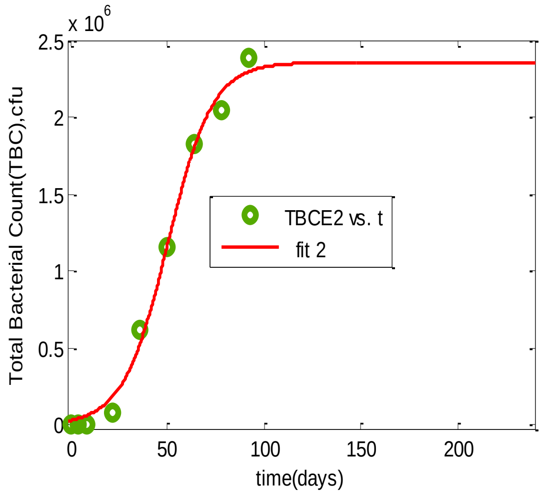

Fig 8: Total Bacterial Count versus time (E2) vs time

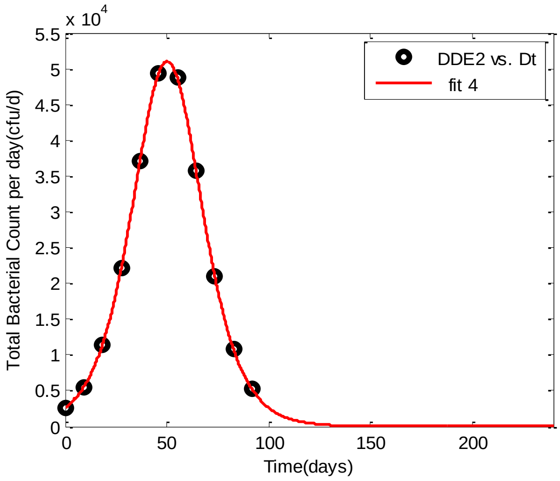

Fig 9: Total Bacterial Count versus time (E2) vs time

Table 2: Coefficients and Statistical Goodness of it for BCE1 to TBCE10

Experiment	**R^2**	**R^2Adj**	**Ultimate (U)**	**Peak values**
TBCE1	0.9913	0.9860	F(209) = 2.883e^6	F(51)=58,172.6
TBCE2	0.9942	0.9907	F(207) = 2.355e^6	F(50) =50974.2
TBCE3	0.9824	0.9718	F(318) = 2.3125e^6	F(318) = 2,3125e^6
TBCE4	0.9824	0.9718	F(306)=2.31054e^6	F(73.6) = 29651.2
TBCE5	0.9880	0.9808	F(235)=2.31228e^6	F(55.2) = 41245.6
TBCE6	0.9184	0.8694	F(210)=1.53988e^6	F(55.2) = 30283.7
TBCE7	0.9495	0.9191	F(285)=1.09932e^6	F(55.2) = 16497.6
TBCE8	0.8611	0.7777	F(179)=1.51585e^6	F(55.2) = 39076.3
TBCE9	0.9836	0.9738	F(273)=1.67858e^6	-
TBCE10	0.9717	0.9547	F(288)=1.00733e^6	F(51) = 15682
TBCE31	0.9688	0.9500	F(393) = 1602.91	F(393) = 14.03

Figure 4 and 5 shows the twin model (biomass increase) curve fitting plot of TBCE1 versus time. In Figure 4, the twin model fitted the biomass experimental data with R^2 of 0.9913 and also predict an ultimate microbial court available for the experiment to be

$2.883e^6$cfu/g on day 209 while Figure 5 shows the microbial daily rate plot which gives a biomass peak value of 58172.6cfu/g on day 51, when the microbes fed most on the contaminants during the experiments. The coefficients and statistical goodness of fit are shown in Table 2.

Figure 6 and 7, curve fits the substrate reduction of TPHE2. In Figure 6, the curve fitting is done within the experimental data with R^2 of 0.9841 while Figure 7 predicts the curve far beyond the experimental data with the same R^2. At DPR 1000mg/kg recommended value of TPH limit in soil the remediation will take 1360 days (3.72years).

Figure 8 and 9 curve fits total bacteria count increase. In figure 8 the natural curve of sigmoidal curve give an ultimate microbial value of F(207) = $2.355e^6$ with R^2 of 0.9942 while in Figure 9, the rate change plot gives a microbial peak of F(50) = 50974.2 with the same R^2.

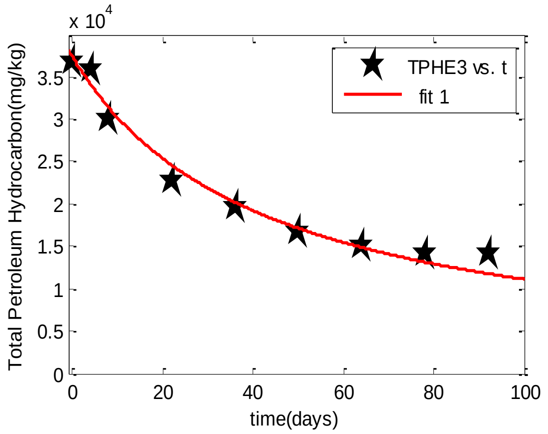

Fig 10: Total Petroleum Hydrocarbon (E3) vs time

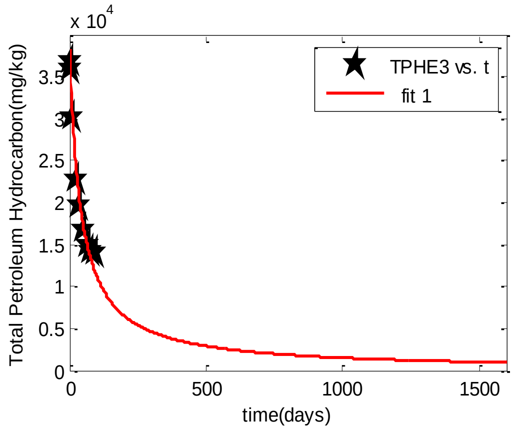

Fig 11: Total Petroleum Hydrocarbon (E3) vs time

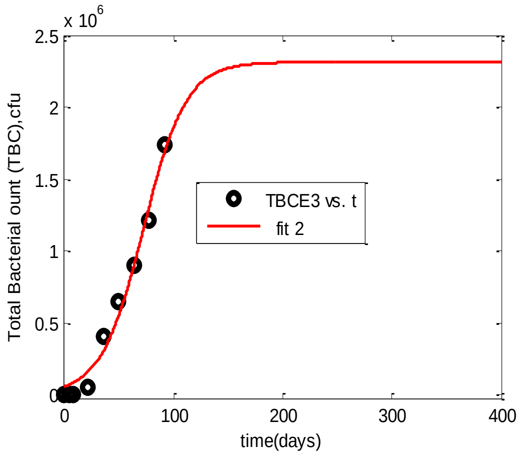

Fig 12: Total Bacterial Count (E3) vs time

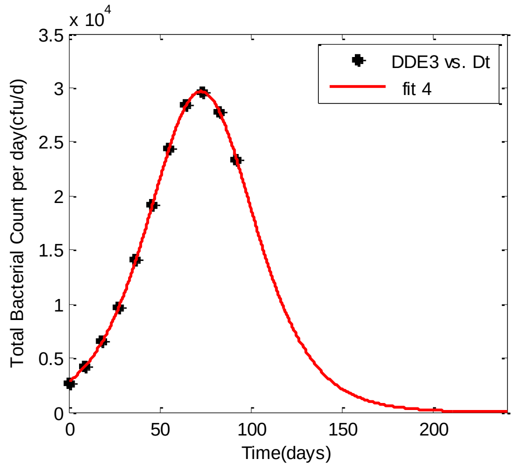

Fig 13: Total Bacterial Count (E3) vs time

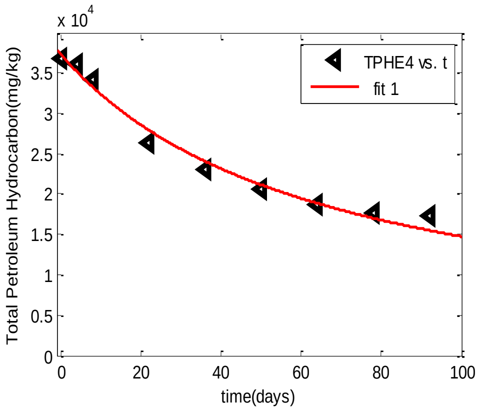

Fig 14: Total Petroleum Hydrocarbon (E4) vs time

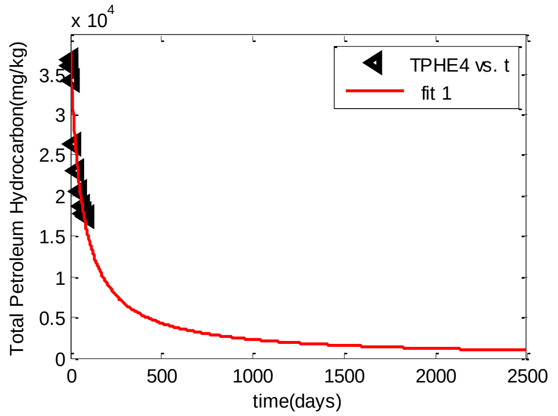

Fig 15: Total Petroleum Hydrocarbon (E4) vs time

In Figure 10 and 11, a plot of substrate reduction was done for TPHE3. Figure 10 shows a plot within the experimental values which gave $R^2 = 0.9772$ while Figure 11 is a predictive plot. The plot pushes the profile far beyond the experimental plot to see its behaviour in future. When projected to the future it took 1512 days (4.14years) to remediate the polluted soil to the DPR recommended value of 1000mg/kg limit of TPH in soil. In Figure 12 and 13 we see the curve fitting of total biomass count. Figure 12 plots a natural sigmoidal of TBCE3 with R^2 of 0.9824 and ultimate value of F(318) = 2.3125e^6 but a daily rate plot of figure 13 gives a peak of F(73.6) = 29647.2.

In Figures14 and 15, substrate reduction plots were carried out for TPHE4. Figure 14 is an exponential decline curve within the experimental results. While, Figure 15 is a predictive curve pushing the profile far beyond the experimental values to see the future with the same R^2. At DPR 1000mg/kg recommended value, the remediation will take 2345dyas (6.43years). However, Figure 16 and 17reported the plot of total biomass count increase, TBCE4 versus time and gave an R^2 of 0.9824 which predicts an ultimate microbial value of F(306) = 2.31054e^6 while its daily rate plot gives a peak of F(73.6) = 29651.2.

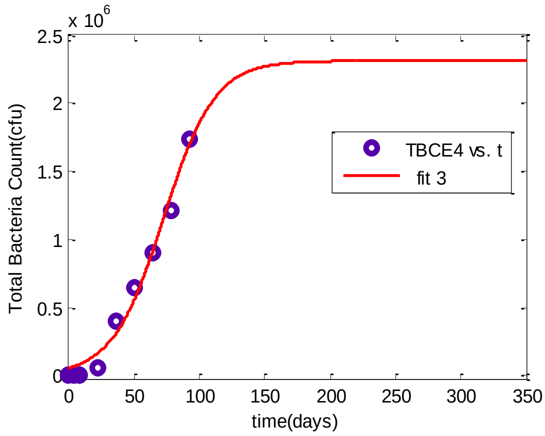

Fig 16: Total Bacterial Count (E4) vs time

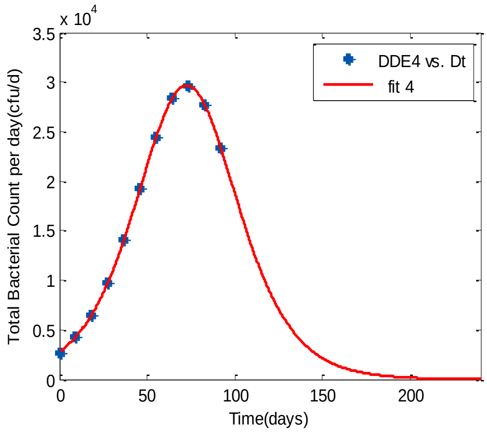

Fig 17: Total Bacterial Count (E4) vs time

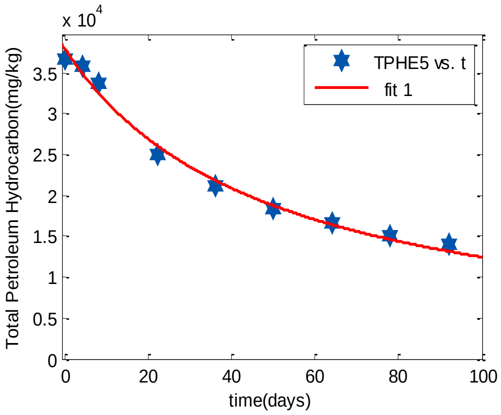

Fig 18: Total Petroleum Hydrocarbon (E5) vs time

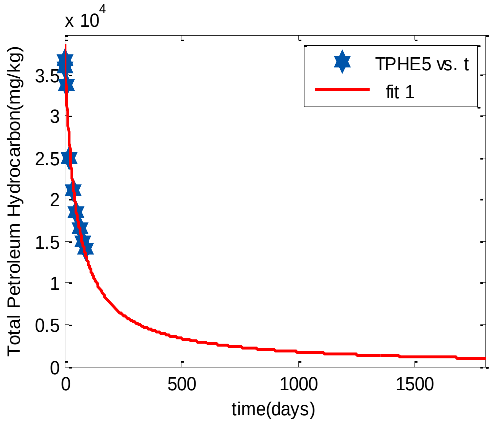

Fig 19: Total Petroleum Hydrocarbon (E5) vs time

In Figures 14 and 15, substrate reduction plots were carried out for TPHE4. Figure 14 is an exponential decline curve within the experimental results. While, Figure 15 is a predictive curve pushing the profile far beyond the experimental values to see the future with the same R^2. At DPR 1000mg/kg recommended value, the remediation will take 2345dyas (6.43 years). However, Figure 16 and 17reported the plot of total biomass count increase, TBCE4 versus time and gave an R^2 of 0.9824 which predicts an ultimate microbial value of F(306) = 2.31054e^6 while its daily rate plot gives a peak of F(73.6) = 29651.2.

Figure 18 and 19, are plots of substrate reduction: in Figure 18 a plot of substrate reduction within the experimental value of TPHE5 with time is made and having R^2 of 0.9909, while in Figure 19 a predictive total reduction is made with the plot pushing the time far above the experimental values to see the future behaviour with the same R^2. At DPR 1000mg/kg of TPH in soil the remediation will take 1761dyas (4.82years). In Figure 20, a natural plot of total bacterial count was made with R2 of 0.9889. It gave us an ultimate microbial value of F(235) = 2.31229e6 while the daily rate plot of figure 21 gave a microbial peak of F(55.2) = 41245.6.

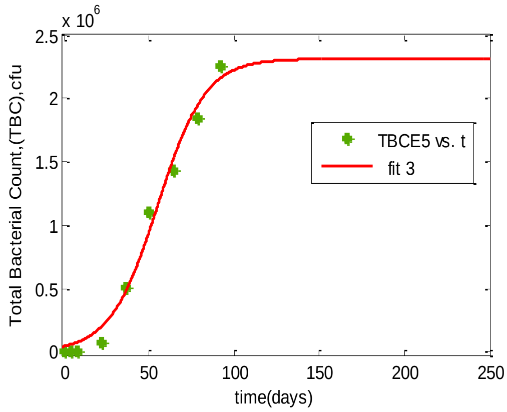

Fig 20: Total Bacterial Count (E5) vs time

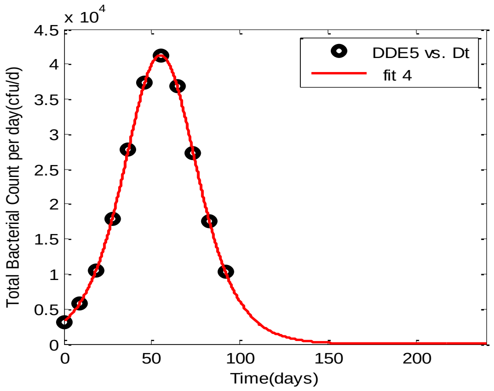

Fig 21: Total Bacterial Count (E5) vs time

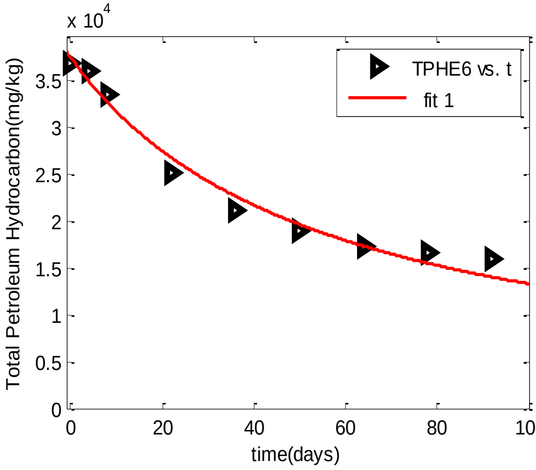

Fig 22: Total Petroleum Hydrocarbon (E6) vs time

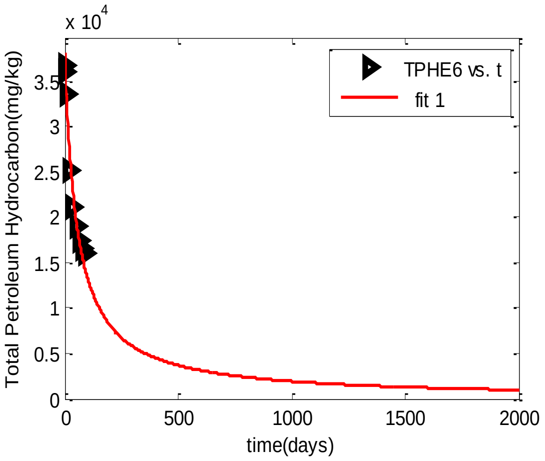

Fig 23: Total Petroleum Hydrocarbon (E6) vs time

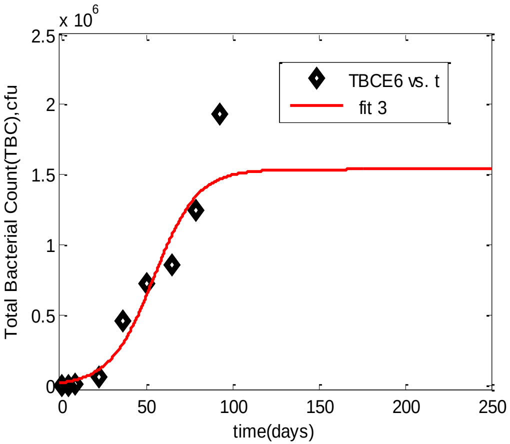

Fig 24: Total Bacterial Count (E6) vs time

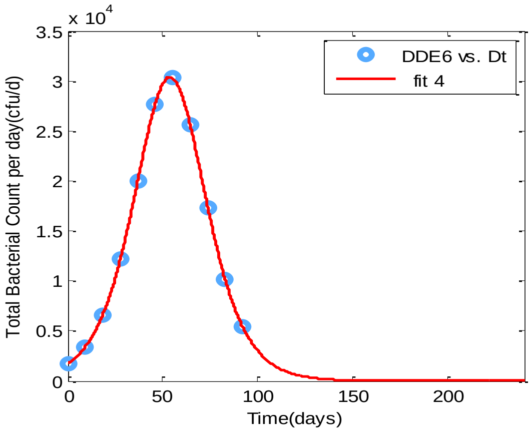

Fig 25: Total Bacterial Count (E6) vs time

In Figure 22 and 23, the substrate reduction plot again is made. In Figure 22, a substrate plot of TPHE6 is made within the experimental values and giving an R2= 0.9783, while in Figure 23 a predictive plot of substrate reduction of TPHE6 with time and the same R2 were made. The plot pushes into the future to know the behaviour of the curve outside the experimental curve. At DPR recommended value of 1000mg/kg TPH limit in soil, the remediation will take 1963 days (5.37years). In Figure 24, a plot of total bacterial count was made for TBCE6 giving an R2of 0.9184 and predicts an ultimate microbial value of F(210) = 1.53988e6 but the daily rate plot of figure 25 gave a peak of F(55.2) = 30283.7.

In Figure 26 and 27, a substrate reduction plot was made for TPHE7 within the experimental value giving an R^2 of 0.9753. While in Figure 27, a predictive curve that pushes into the future of time to know the behaviour of the curve was made. With the same R^2 and DPR limit of 1000mg/kg of TPH in soil, the length of time of remediation was put at 2715days (7.43years). In Figure 28 a plot of total bacterial count increase was made with R^2 = 0.9495 and predicts an ultimate microbial value of F(285) = 1.0992e^6.The daily rate plot of Figure 29gives a microbial value peak value of F(55.2) = 16497.6.

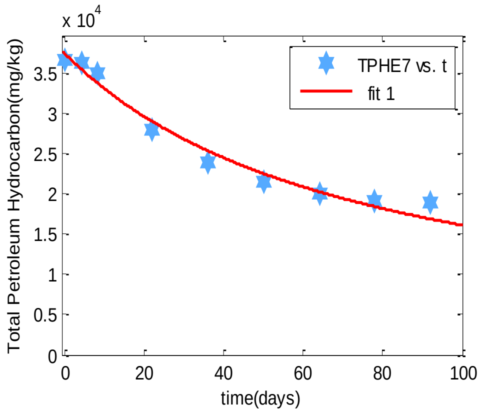

Fig 26: Total Petroleum Hydrocarbon (E7) vs time

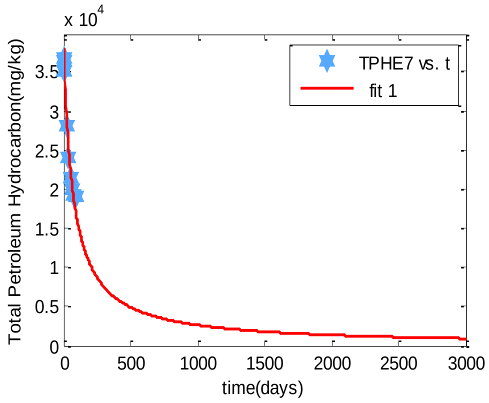

Fig 27: Total Petroleum Hydrocarbon (E7) vs time

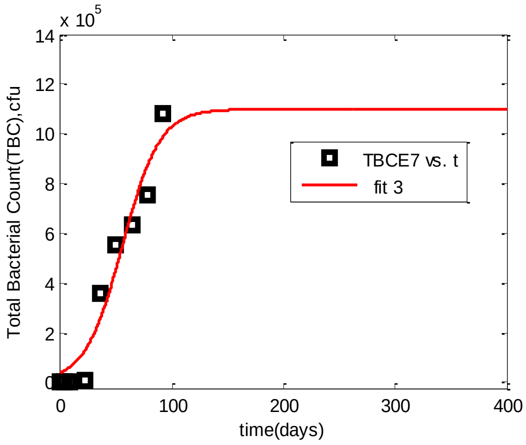

Fig 28: Total Bacterial Count (7) vs time

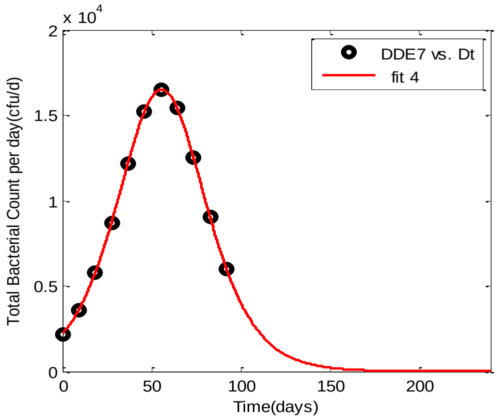

Fig 29: Total Bacterial Count (7) vs time

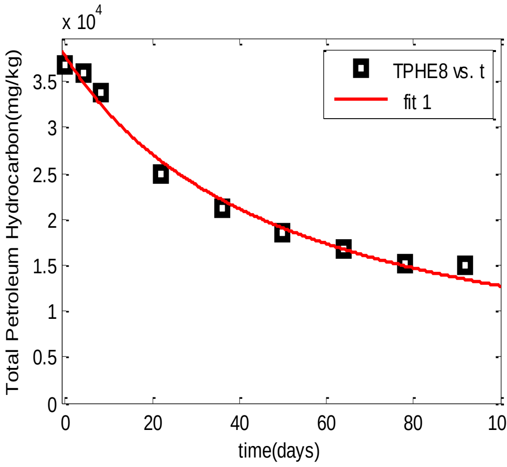

Fig 30: Total Petroleum Hydrocarbon (E8) vs time

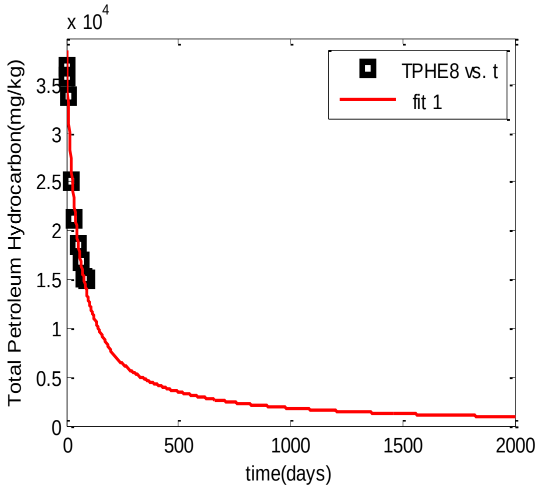

Fig 31: Total Petroleum Hydrocarbon (E8) vs time

In Figures 30 and 31, the substrate reduction plot of TPHE8 was made within experimental data giving $R^2 = 0.9872$. In Figure 31 a predictive substrate reduction plot with the same R^2 of TPHE8 was made. The plot pushing into the future to predicted 1835 days (5.03 years) to remediate the polluted soil to the DPR 1000mg/kg limit of TPH in soil. In Figure 32, a plot of total bacterial count TBCE8 against time was made giving R^2 of 0.8611 and predicts an ultimate microbial value of F(179) = 1.585e⁶. In Figure 33, the microbial daily rate profile gave a peak of F(55.2) =39076.3.

In Figures 34 and 35, exponential substrate reduction was plotted for TPHE9. In Figure 34, a plot of substrate reduction within the experimental value gave R^2 of 0.9909 while same plot pushed to the future in Figure 35 showed a display of what will happen in the future experimental data with same R^2. At DPR 1000mg/kg recommended value of TPH limit in soil, the remediation time will be 2408days (6.60years). In figure 36, the plot of total bacterial count TBCE9 against time was made. In Figure 36, the plot fitted well with R^2 of 0.9985 (within the data points) but it cannot predict an ultimate, hence experiment 9 is a bad experiment. The model was pushed beyond the experimental values into the far future, the result shows that the model cannot taper out asymptotically and therefore cannot predict.

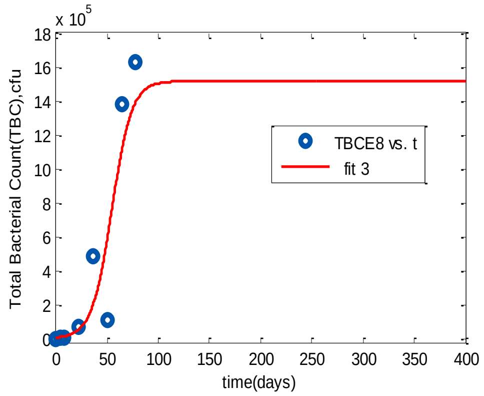

Fig 32: Total Bacterial Count (E8) vs time

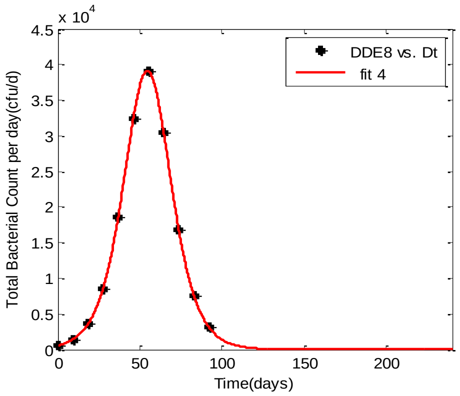

Fig 33: Total Bacterial Count (E8) vs time

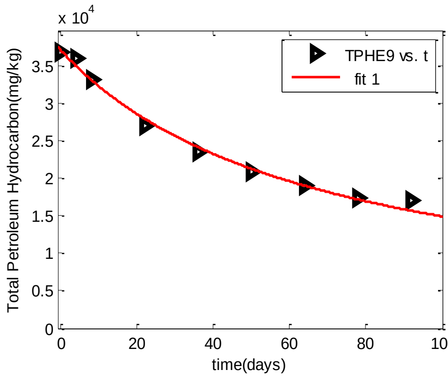

Fig 34: Total Petroleum Hydrocarbon (E9) vs time

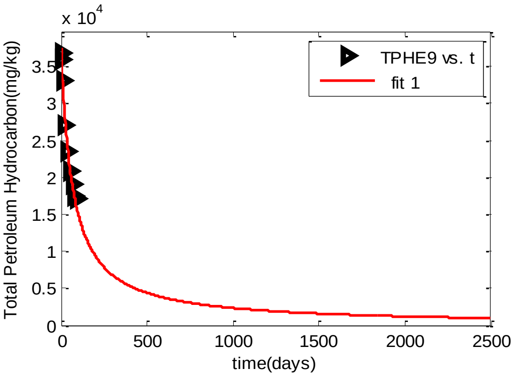

Fig 35: Total Petroleum Hydrocarbon (E9) vs time

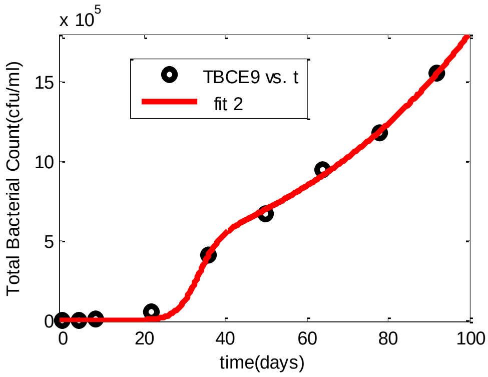

Fig 36: Total Bacterial Count (E9) vs time

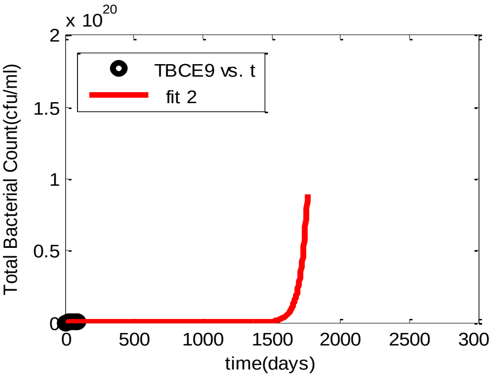

Fig 37: Total Bacterial Count (E9) vs time

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In Figures 38 and 39, the substrate reduction plot was made for TPHE10 giving an R^2 of 0.9499and when pushed beyond the experimental time in Figure 39 at DPR value of 1000mg/kg, recommended value of TPH limit in soil, the remediation period will be 2955day (8.09years). In Figure 40, a biomass increase was shown in the plot (TBCE10 versus time) which gave an R^2 of 0.9717. The model tappers out asymptomatically to give an ultimate microbial value of F(288) = 1.00733e^6 while the daily rate of figure 41 gives a microbial peak of F(51) = 15682.

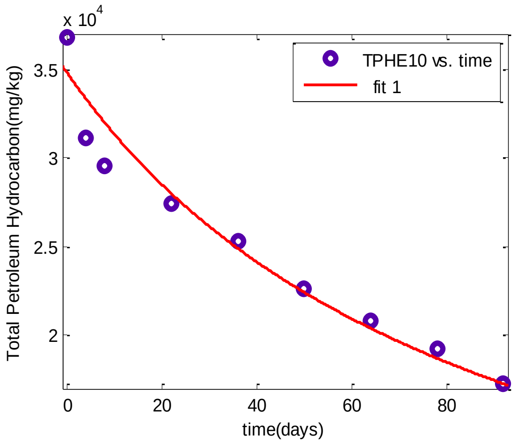

Fig 38: Total Petroleum Hydrocarbon (E10) vs time

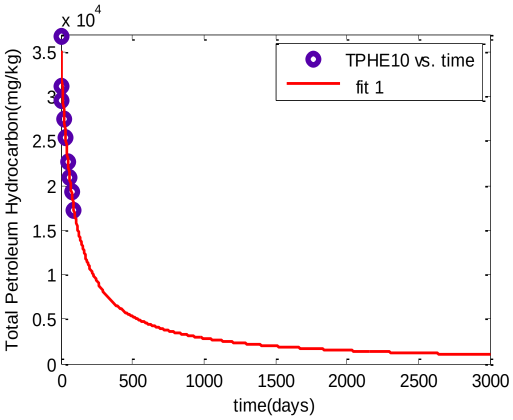

Fig 39: Total Petroleum Hydrocarbon (E10) vs time

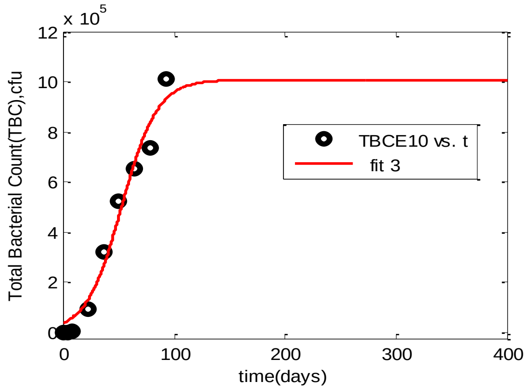

Fig 40: Total Bacterial Count (E10) vs time

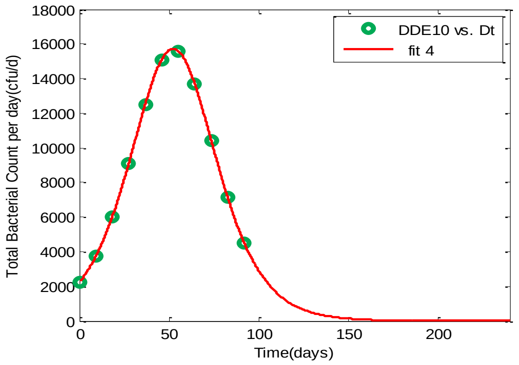

Fig 41: Total Bacterial Count (E10) vs time

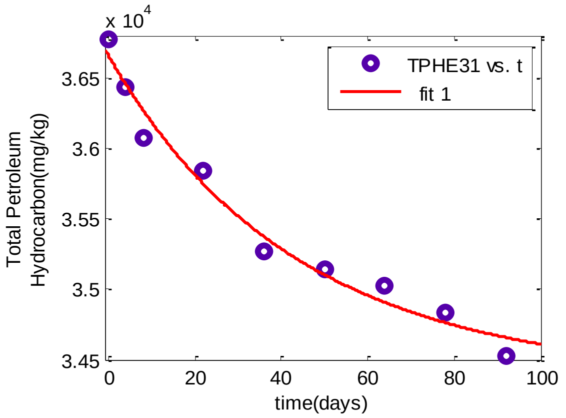

Fig 42: Control; Total Petroleum Hydrocarbon (E31) vs time

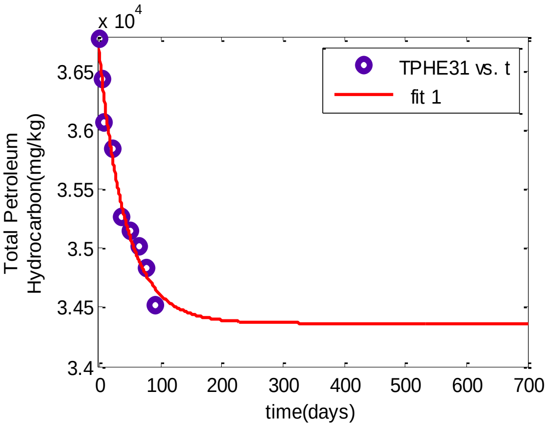

Fig 43: Control; Total Petroleum Hydrocarbon (E31) vs time

Figures 42 and 43 show the curve fitting profile of the substrate reduction model on the Experimental data, E31 (control experiment). The substrate reduction model made a good fit to the experimental data. The fit gave R-squared value of 0.9764. Hence, the model explains over 97% of the variabilities observed in the experimental data. Figure 43 is a predictive profile of the substrate reduction model outside the experimental data. The model gave a poor prediction of a remediation time of 506days to reduce the substrate concentration of the soil from 36775.682mg/kg to 34 775.692mg/kg. The model couldn't predict when the remaining substrate concentration will be 1000mg/kg (DPR acceptable limit of TPH in soil). This poor prediction was due to the fact that the control experiment was not amended with any biostimulant as it proceeded on natural attenuation. Figures 44 and 45 are curve fitted profiles of the microbial increase model on the plotted scatter diagram of the Total bacteria count experimental data. The model fitted the experimental data with an R-squared value of 0.9688 indicating that the model explains over 96% of the variability observed in the experimental data. The model curve fitting in figure 44, predicted an ultimate microbial value of F(393) = 1602.91 while the daily rate plot in Figure 45 gives a microbial peak value of F(27.6) = 1403.

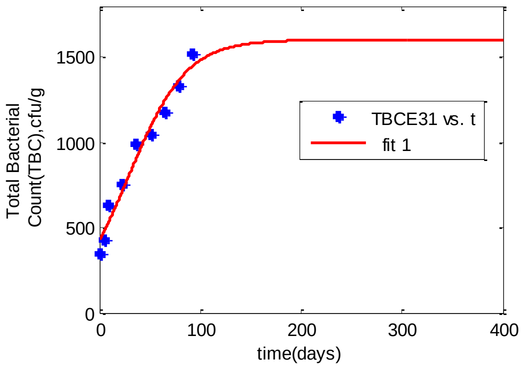

Fig 42: Control- Total Petroleum Hydrocarbon (E31) Vs time

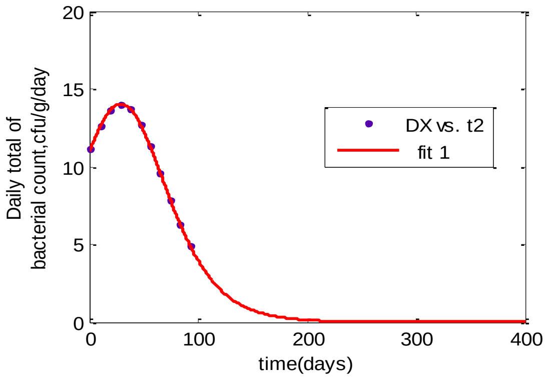

Fig 45: Control- Total Bacteria Count (E31) Vs time

CONCLUSION

In this study the bioremediation of crude oil sludge polluted soil was investigated using inorganic and organic fertilizers such as NPK, Cow dung and moringa *oleifera* leaf powder which is being introduced for the first time as biostimulant. The conventional biostimlants as well as the *moringaoleifera* leaf powder all enhanced the degradation of the crude oil sludge impacted soil. The twin model which was developed in this study validated the experimental data. The results of the model can be used for monitoring and predicting bioremediation in the Niger Delta. The best experiment is experiment E1. The twin model (substrate reduction and biomass increase) fitted the experiment E1 with R^2 value of 0.9794 and 0.9913 respectively. The twin model curve fitting predicted an earliest remediation time of 3.4years with respect to experiment E1 in comparison to other experiments with higher remediation time. Hence experiment E1 is the best experiment. It is recommended that further work be carried out *in-situ* with a view to comparing results with the present study which was carried out *ex-situ.*

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